



# Nutrient stabilization and heavy metal reduction in organic wastes using *Eisenia fetida* (Savigny) and *Perionyx excavatus* (Perrier)

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## Abstract

Concern about waste production, conversion, or disposal has been the main issue every society or government emphasizes. The viability of using earthworms to mitigate environmental pollution through waste biomass and the remediation of polluted soil is investigated scientifically. The present study probes the effect of *Eisenia fetida* and *Perionyx excavatus* in manure production, the transformation of nutrients, and heavy metal reduction in four treatments [kitchen scrap (KS), cow dung (CD), rice straw (RS), and mixed substrate (MX)]. The experiment was conducted in pre-decomposed wastes for 60 days in a 3:2 ratio of organic waste and cow dung experimental design. Twenty earthworms were inoculated in each replicated setup and subjected to vermicomposting. Results show that manure productivity (g) was highest in control, followed by *P. excavatus* and *E. fetida*, indicating high substrate degradation in the presence of earthworms. The reduced manure production (g) during vermicomposting indicates that earthworms ingested, degraded, and transformed waste biomass. Organic carbon (OC), total nitrogen (TN), and carbon:nitrogen (C:N) ratio declined, while available phosphorus (Av. P) and available potassium (Av. K) concentrations increased substantially. High increased percentage of Av. P in manure produced from *P. excavatus* (29.93%) and *E. fetida* (36.13%) pot was observed. Similarly, Av. K showed an increasing percentage of 24.37% and 27.42% in *P. excavatus* and *E. fetida*. Also, earthworm activities lead to a significant reduction in heavy metal concentration. In *E. fetida*, *P. excavatus*, and control high percentage reduction of copper (Cu) ( $67.52 \pm 5.98\%$ ,  $67.51 \pm 4.32\%$ , and  $48.77 \pm 8.62\%$ ), iron (Fe) ( $28.88 \pm 13.03\%$ ,  $36.9 \pm 22.11\%$ , and  $25.4 \pm 14.02\%$ ), manganese (Mn) ( $94.43 \pm 0.1\%$ ,  $84.1 \pm 0.95\%$ , and  $62.24 \pm 3.56\%$ ), and zinc (Zn) ( $22.81 \pm 3.95$ ,  $27.1 \pm 7.36$ , and  $11.87 \pm 5.71\%$ ) were observed. The reduced waste biomass enriched macronutrients and minimized heavy metals concentration indicate the suitability for selected earthworm species to address environmental issues related to waste biomass.

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## 1 Introduction

Solid waste management is one of the most challenging environmental issue faced by all nations today. Several strategies are adopted to valorize solid organic waste and agricultural residues. These are composting, production of the board, binder-less board paper, or converting this organic waste to clean fuels and petrochemical substitutes via pyrolysis. Organic waste may also be recycled by enhanced hydrolyzed urine, hydrolysis to sugar, which may be fermented to give bioethanol (Alemayehu et al., 2022a, 2022b; Fahmy et al., 2020, 2017; Fahmy & Mobarak, 2013). Effective management of solid waste is essential to build a sustainable society and contributes to the mitigation of environmental pollution. However, it is one of the most ignored and substandard services being provided by the government and local authorities (Ostad-Ali-Askari, 2022; Letcher & Vallero, 2019). Biomass from agriculture waste, household products, and livestock waste has potential benefits, especially for the rural economy. Without recycling, waste disposal to water bodies, agricultural soil, and open field dumping creates a burden of heavy metal contamination, nutrient loss, atmospheric pollution, and health hazards. Therefore, it is imperative to assess the waste management system considering the three core waste management hierarchy systems (3CWMHS), prevention and reduction, recycling and recovery of energy, and alleviating the health risk to residents (Doaemo et al., 2021).

The traditional aerobic composting of organic waste leads to the loss of nitrogen through ammonia gas and nitrogen oxides, reducing the fertilizing value of manures and contributing to the Greenhouse Gases (GHGs) in the atmosphere (Javadinejad et al., 2019; Zhu-Barker et al., 2017). However, these limitations can be bridged by vermicomposting, a reliable, efficient, and environmentally friendly method of waste management, and this is gaining interest among many researchers across the globe. Some studies suggest that vermicomposting also generates GHGs into the atmosphere, but recent studies have shown that a controlled process reduces emissions (Rini et al., 2020). During vermicomposting, earthworms ingest, grind, and digest the waste, and its gut-associated microorganisms play a significant role in nutrient transformation (Sun et al., 2020; Edwards & Bohlen, 1996). The joint action of earthworms and microorganisms convert waste to high-quality manure with a rich amount of nutrients such as nitrogen, phosphorus, potassium, and calcium available for plant absorption (Rajkhowa et al., 2015). Besides, the product (manure) of vermitechnology is known to harbor a higher number of phosphate solubilizing bacteria (PSB) which further enhances phosphorus availability (Lirikum et al., 2022). Vermicomposting changes the bacterial diversity prominently and reduces heavy metals from organic waste (Wang et al., 2017). However, depending on the substrate used, compost and vermicompost generally still contain heavy metals. Hence it is essential to quantify heavy metal content to avoid soil contamination, metal toxicity to soil biota, bio-concentration in crop yield, and meet the legal regulations.

India is one of the fastest-growing developing countries in the world, but with the advancement in the growing economy, serious measures need to be addressed as far as sustainability and circular economy is concerned. Considering the large amount of agricultural (about 350 million tonnes) and other domestic waste generation in India, conversion of waste into fertilizing manure through eco-friendly management is shallow (ICAR, 2020). The enormous volume of waste biomass are either disposed of in the agricultural fields, burnt on site, or

disposed of along the roads or railway tracks which causes environmental problems and loss of valuable nutrients in the plant biomass (Thomas et al., 2019). Hence, an environmentally friendly approach to solid waste containment, especially in urban areas, has become vital.

Earthworms are the most abundant animals in the terrestrial ecosystem and are considered keystone species (Li et al., 2022); exploration of multiple species with better efficiency in waste management needs to be carried out for a cleaner and healthier ecosystem. Some of the standard features upon which earthworms are considered suitable for vermicomposting include- Surface or litter dwelling, ability to colonize in an organic-rich substrate, high rate of organic matter consumption, digestion and assimilation, adaptations to a wide range of environmental conditions, and high reproduction and short life cycle (Dominguez & Edwards, 2011). The biology and physiology of epigeic earthworms *E. fetida* and *P. excavatus* fulfill the above-listed characteristics; these earthworm species have the potential to be used for minimizing waste disposal problems (Hasan et al., 2022). *E. fetida*, also known as red-wrigglers, are commercially available earthworms used for vermicomposting, while *P. excavatus* or blue Indian worm is another preferred species for vermiculture in the Indian subcontinent. However, comparatively, waste degradation efficiency and nutrient conversion potential of *P. excavatus* in a tropical country like India is minimal.

Recently vermicomposting has been studied using many different type of wastes, including agricultural and food waste (Wang et al., 2022), coir pith (Jayakumar et al., 2022), and eucalyptus leaves (Bhagat et al., 2022). In India, several researchers have experimented the nutrient recovery process through vermicomposting of domestic waste and weeds using *E. fetida* and *P. excavatus* (Kaladhar & Srinivasan, 2022; Mago et al., 2021; Devi & Khwairakpam, 2020). In Nagaland (India), scanty records of the characterization of macronutrients obtained from vermicomposting of agricultural wastes using *E. fetida* are available (Borang et al., 2016; Chatterjee et al., 2016) however, efficiency of *P. excavatus* in nutrient stabilization and heavy metal remediation through vermiculture is lacking. Therefore to abide by the initiatives and governmental regulations to implement the practice of organic farming practices, waste management, and mitigation of pollutants in the environment, exploitation of these potential macroinvertebrates (*E. fetida* and *P. excavatus*) is vital.

In Nagaland, situated in the easternmost corner of India, agriculture is a significant economic activity, out of which a large amount of domestic and agricultural waste are generated. But knowledge of wastes' bioconversion using earthworms into nutrient-rich manure and reduced heavy metal toxicity is minimal. Therefore, the present study, being the first of its kind, was conducted to assess manure production and changes in physicochemical parameters like pH, organic carbon (OC), Total nitrogen (TN), Available phosphorus (Av. P), and Available potassium (Av. K) using locally available earthworm *P. excavatus* and *E. fetida* in an aerobic vermicomposting set up. Also, mitigation of heavy metal viz., Iron (Fe), copper (Cu), Manganese (Mn), and Zinc (Zn), in organic biomass such as kitchen waste, rice straw, cow dung, and a mixture of all organic waste was tested in the present study.

## 2 Materials and methods

### 2.1 Earthworm species

The experiment was conducted using two earthworm species, i.e., *P. excavatus* and *E. fetida*. The *P. excavatus* was collected from the Mingkong forest (Fig. 1) of Nagaland (26° 21' 50.18" N and 94° 33' 37.20" E) and authenticated at ZSI (Zoological Survey of India),

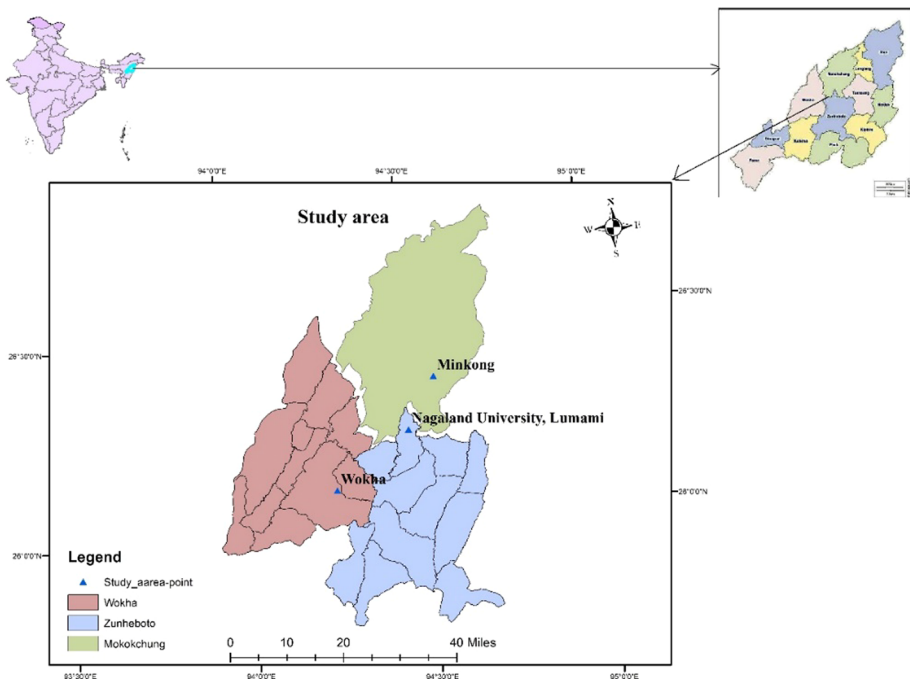
Kolkata. The commercially available species *E. fetida* was procured from a local vermicomposting farm, Wokha town (26° 05' 26.82" N and 94° 15' 31.33" E), Nagaland, India. Both species were maintained separately in the vermicomposting chamber as stock culture using cow dung as food substrate for subsequent experiments.

## 2.2 Collection of raw materials

Rice straws were collected from five months old Jhum field (26° 13' 42.89" N and 94° 28' 24.70" E) near Nagaland University Lumami, Zunheboto, (Fig. 1), and brought to the laboratory. The required amount of domestic kitchen scraps were collected daily for ten days from the Kamnoi, Research Scholar Hostel (Nagaland University, Lumami). Before setting up the experiment, rice straws and kitchen scraps were chopped into smaller pieces of 1–2 inches and pre-decomposed for 15 days with regular sprinklings of water to facilitate decomposition. From a local farm, urine-free cow dung of *Bos indicus* (Vechur) fed with green plants was collected. Fresh cow dung generally generates heat and toxic compounds that cause mortality to the earthworm. Therefore the collected dung was pre-decomposed for 15 days.

## 2.3 Experimental design

The vermicomposting was performed in a square plastic container of 20 × 20 × 16 cm. For the vermibed, a combination of rice straw, kitchen waste, and mixed substrate was prepared



**Fig. 1** Map shows earthworms' collection area and raw materials under Mokokchung, Wokha, and Zunheboto district, Nagaland, India

with cow dung at a 3:2 (w/w) ratio. Since the earthworm belongs to the epigeic species category and requires high moisture to establish its populations, the vermibed was maintained at 70–80% moisture (Table 1). Each experimental setup (*P. excavatus*, *E. fetida*, and control) contained 2 kg of raw materials (on a dry weight basis) and was established in triplicates. The materials in all the setup were allowed to decompose for three weeks to control the moisture and eliminate volatile toxic gases for better earthworm and microorganism activity (Sharma & Garg, 2017; Yadav & Garg, 2016). Due to its high organic matter and rich nutrients, cow dung was used as a standard medium for all the treatments (Reinecke et al., 1992; Reinecke & Hallatt, 1989).

In each setup, 20 healthy clitellated *P. excavatus* and *E. fetida* (average biomass  $3.29 \pm 0.03$  g and  $4.08 \pm 0.41$  g) were inoculated except control. The vermibeds were kept in the dark, and the adequate moisture was maintained by sprinkling water. The vermicomposting pot were covered with jute bags to prevent moisture loss and turned once a week. After 60 days, the vermicompost was collected from all the experimental setups. The collected samples were air-dried and stored in airtight plastic bags for a comparative study of physicochemical parameters. Final weight of the vermibed was weighed at the end of the experiment, and comparisons were made among the treatments.

## 2.4 Physicochemical analysis

Initial substrates were dried, crushed, and sieved through a 1 mm mesh size sieve and analyzed for pH, OC, TN, Av. P, Av. K, Cu, Fe, Mn, and Zn.

pH was measured using a digital pH meter at 1:25 sample-water solution. The modified wet oxidation method initially described by Walkley & Black (1934) was used to determine OC. Samples were digested with potassium dichromate ( $K_2Cr_2O_7$ ) using sulphuric acid ( $H_2SO_4$ ), and digested samples were further titrated with ferrous ammonium sulphate ( $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ ) in the presence of diphenylamine ( $C_{12}H_{11}N$ ) as an indicator. The endpoint, indicated by green color, was noted and calculated for organic carbon (%).

TN was determined using Kel plus instrument (Pelican equipment- Classic- DX VAT-E). Through digestion, amino nitrogen in the sample is converted into ammonium radicals

**Table 1** Experimental setup of vermicomposting using different substrate combinations and earthworm species

Substrate used	Earthworm species	Feedstock composition ratio
Kitchen scrap (KS)	<i>P. excavatus</i>	KS + CD (3:2)
Cow dung (CD)	<i>P. excavatus</i>	CD only
Rice straw (RS)	<i>P. excavatus</i>	RS + CD (3:2)
Mixed (MX)	<i>P. excavatus</i>	MX + CD (3:2)
Kitchen scrap (KS)	<i>E. fetida</i>	KS + CD (3:2)
Cow dung (CD)	<i>E. fetida</i>	CD ONLY
Rice straw (RS)	<i>E. fetida</i>	RS + CD (3:2)
Mixed (MX)	<i>E. fetida</i>	MX + CD (3:2)
Kitchen scrap (KS)	No earthworms	KS + CD (3:2)
Cow dung (CD)	No earthworms	CD only
Rice straw (RS)	No earthworms	RS + CD (3:2)
Mixed (MX)	No earthworms	MX + CD (3:2)

KS kitchen scrap; CD cow dung only; RS rice straw, MX mixture of kitchen scrap, rice straw and cow dung

in the presence of strong acid ( $\text{H}_2\text{SO}_4$ ) aided by potassium sulphate ( $\text{K}_2\text{SO}_4$ ) and copper sulfate ( $\text{CuSO}_4$ ) as a catalyst. Further, separation and isolation of nitrogen from the digestion tube were processed through distillation in the presence of sodium hydroxide ( $\text{NaOH}$ ), during which ammonium radicals get converted into ammonia gas which was collected by trapping in 4% boric acid. At last, TN determination was done by titrating with 0.1 N hydrochloric acid ( $\text{HCl}$ ) in the presence of methyl red and bromocresol green (8:10) as an indicator.

Av. P was determined spectrophotometrically (Systronic spectrophotometer-166) using a modified form of Bray & Kurtz (1945). The bound form of phosphorus and acid-soluble phosphorus was extracted using a bray reagent containing 0.03 N ammonium fluoride ( $\text{NH}_4\text{F}$ ) and 0.025 N  $\text{HCl}$  acid, and the amount of phosphorus was determined by the intensity of blue colour development when treated with a molybdate-ascorbic acid reagent.

Av. K was determined by a modified procedure of the one initially described by Hanway & Heidel (1952). Neutral ammonium acetate ( $\text{C}_2\text{H}_4\text{O}_2\cdot\text{H}_3\text{N}$ ) solution was used as extracting solution for exchangeable potassium ions, which was further determined by a flame photometer (Systronic flame photometer-130).

Heavy metals (Fe, Cu, Mn, and Zn) were estimated following the DTPA method (Lindsay & Norvell, 1978) using Atomic absorption spectrophotometer (SHIMADZU CORP, AA-6880). Soil samples were mixed with extractant containing 0.005 M diethylenetriaminepentaacetic acid (DTPA), 0.1 M triethanolamine ( $\text{C}_6\text{H}_{15}\text{NO}_3$ ), and 0.01 M Calcium chloride ( $\text{CaCl}_2$ ) at a 1:2 ratio (10 g of soil in 20 mL of extractant) and shaken for 2 h at 25 °C. Samples were filtered through Whatman no.1 filter paper, and against the standard solutions for each metal, the filtrates were measured at AAS using the respective lamp.

## 2.5 Statistical analysis

The data is presented in mean  $\pm$  SD. Analysis of variance (One-way ANOVA) at 95% intervals ( $p < 0.05$ ) was conducted to find the mean significant difference among treatment. While two-way ANOVA was conducted to find the effects of the interaction of independent variables (treatment and substrate) and also the main effects on final soil nutrient parameters. Each test was followed by a multiple comparisons test (Tukey test) to find the mean significant difference between the variables. The statistical analysis was conducted using IBM SPSS- 22 software.

## 3 Results and discussion

### 3.1 Substrate degradation

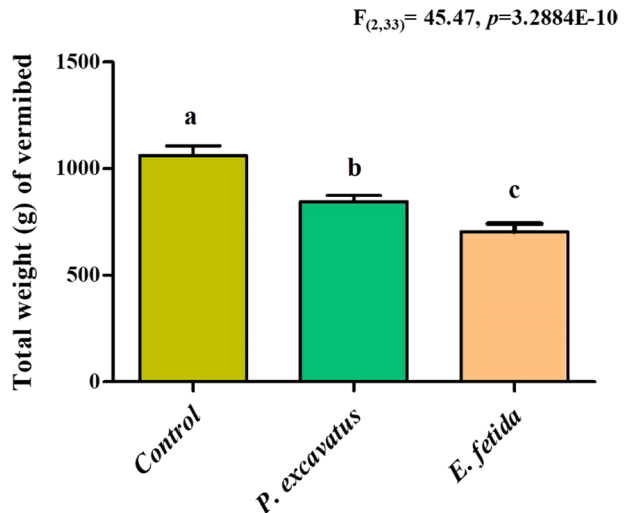
The final products of the vermicompost were dark, homogeneous, and finely textured that could retain more moisture. The total weight of vermibed (g) in the presence and absence of earthworms were significantly different ( $F_{(2, 33)} = 45.17, p < 0.05$ ), indicating better substrate degradation in the earthworm-treated pot. Higher rate of substrate decomposition was found in *E. fetida* (64.78%), followed by *P. excavatus* (57.53%) and control (46.85%) which resulted into lower weight of total vermibed (Fig. 2). Two-way ANOVA was performed to analyze the effect of earthworm species and substrate used on total manure productions. The results shows that interaction effects of earthworm species and substrates on manure production were insignificant ( $F_{(6, 24)} = 2.09, p > 0.05$ ). But there was significant

main effects of earthworm species ( $F_{(2, 24)}=71.01, p<0.05$ ) and substrate ( $F_{(2, 24)}=4.98, p<0.05$ ) on manure productions. Paul et al. (2011) reported a 70.48% biomass reduction of municipal wastes during vermicomposting using *P. ceylensis*. Similarly, Huntley & Ansari (2021), during vermicomposting of vegetable wastes using *P. excavatus*, the final amount of manure produced was 51.54% (515.45 g out of 1000 g) initial substrate used. Sharma & Garg (2018) suggested that the decomposition rate depends on the efficiency of earthworm species and the nature of organic materials used during vermicomposting.

### 3.2 Changes in macronutrients after vermicomposting

The amount of OC (%) recorded initially in all the substrates ( $23.5 \pm 1.72, 23.13 \pm 1.06, 23.99 \pm 1.43$ , and  $29.92 \pm 1.13$  in KS, CD, RS, and MX, respectively) was reduced subsequently at the end of the experiment (Table 2). In control ( $14.71 \pm 2.73$ ), the mean value of OC was found to be higher, followed by *E. fetida* ( $13.07 \pm 3.1$ ) and *P. excavatus* ( $11.4 \pm 2.74$ ), exhibiting significant differences among the treatments ( $F_{(2, 33)}=3.99; p<0.05$ ). Multiple comparison tests (Tukey's) show mean value of OC in control and *P. excavatus* differ significantly ( $p<0.05$ ) however, no significant difference ( $p>0.05$ ) was observed between the two earthworm species used. Also, depending on the substrate used, OC varies significantly ( $F_{(3, 32)}=3.55, p<0.05$ ) depending on the substrate. Effects of earthworm species and substrate on OC dynamics were analyzed using two-way ANOVA. The results show that the interaction effect of species and substrate has a significant impact on OC dynamics ( $F_{(2, 24)}=6.71, p<0.05$ ). Also, main effect of earthworm species ( $F_{(2, 24)}=11.29, p<0.05$ ), substrates ( $F_{(3, 24)}=9.64, p<0.05$ ) were significant. In the control pot, OC reduction was maximum in KS ( $51.84 \pm 10.62\%$ ) and minimum in CD ( $28.53 \pm 4.73\%$ ). In *P. excavatus* inoculated pot, the reduction of OC was more in CD ( $63.22 \pm 6.91\%$ ), followed by KS ( $59.72 \pm 5.09\%$ ), MX ( $52.58 \pm 1.73\%$ ), and RS ( $43.18 \pm 6.9\%$ ). While in *E. fetida*, the maximum reduction was observed in RS ( $57.94 \pm 1.03\%$ ), followed by MX ( $51.71 \pm 6.94\%$ ), KS ( $51.34 \pm 11.34\%$ ), and CD ( $28.28 \pm 14.64\%$ ) (Fig. 3). The average reduction percentage of OC was maximum in *E. fetida* ( $54.67 \pm 9.31\%$ ) followed by *P. excavatus* ( $47.31 \pm 5.88\%$ ) and control ( $41.12 \pm 3.91\%$ ).

**Fig. 2** Final weight of vermibed in control and earthworm treatment. Mean with different superscripts (a, b, c) among the treatment differ significantly by Tukey's test at a 95% confidence level ( $p<0.05$ )



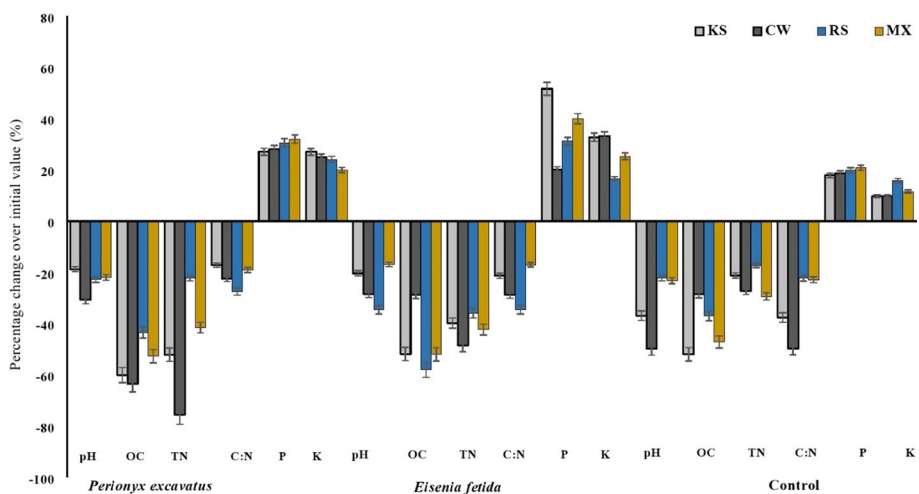


The mean reduction of OC shows significant variations ( $F_{(2,33)}=3.92, p<0.05$ ) among the treatment. Multiple comparison tests show decomposition was significantly higher in *E. fetida* ( $p<0.05$ ) compared to the control, however, no significant difference was observed between the control and *P. excavatus* ( $p>0.05$ ).

During vermicomposting, assimilation and respiratory activity of earthworms, in association with microbes, utilize biodegradable organic matter resulting to a rapid decrease in organic carbon (Lv et al., 2018; Sharma & Garg, 2018) have reported 17–58% of total organic carbon reduction from rice straw, paper waste, and cow dung mixtures during vermicomposting. In contrast, Mago et al. (2021) reported a 40.09–64.06% reduction of organic matter from banana crop waste. Also, the decomposition and humification of carbonaceous materials in the substrate led to reduced OC (Negi & Suthar, 2018). OC reduction could also be attributed to the emission of  $\text{CO}_2$  from vermibin (Lv et al., 2018).

Similar to OC, the initial concentration of total nitrogen (TN%) (Ranging from 1.35 to 2.06%) was considerably reduced in all substrates. Although the variation in TN mainly was observed depending on the nature of the biodegradable waste statistically, no significant ( $F_{(3,32)}=0.86, p>0.05$ ) differences were observed among the substrate. But, on assessing the effects of earthworm treatment, TN reduced significantly ( $F_{(2,33)}=14.94, p<0.05$ ), and multiple comparisons test shows the average amount of TN in *P. excavatus* ( $0.84\pm 0.25\%$  and *E. fetida* ( $0.98\pm 0.1\%$ ) were significantly ( $p<0.50$ ) lower compare to control ( $1.29\pm 0.22\%$ ). However, no significant differences ( $p>0.05$ ) were observed between the two earthworm species. In control, TN varied from 1.12 to 1.5% (MX>CD>KS>RS), while in *P. excavatus* and *E. fetida* TN was recorded between 0.5 and 1.09% (MX>RS>KS>CD) and 0.86–1.07% (MX>CD>KS>RS) (Table 2).

The percentage reduction of TN among different substrates over the initial value was recorded to be 16.75–29.2%, 21.86–75.49%, and 35.82–48.38% in control, *P. excavatus*, and *E. fetida*, respectively (Fig. 3). The average reduction of TN in *P. excavatus* (47.56%), *E. fetida* (41.36%), and control (23.82%) were significantly different ( $F_{(2,33)}=9.2, p<0.05$ ), indicating that nitrogen mineralization depends on the nature of substrate and type of earthworm employed. With the joint action of microorganisms, earthworms bring about



**Fig. 3** Various physicochemical parameter percentage changes over the initial value in the control and earthworm treatment. KS kitchen scrap, CD cow dung, RS rice straw, MX mixed substrate



numerous changes in biodegradable waste ranging from physical, chemical, and biological characteristics. The decline in nitrogen could be due to the utilization of organic matter by heterotrophic microorganisms and the release of ammonia gas. Zhi-Wei (2019) observed a significant reduction in the final vermicomposted organic matter (1.01–0.54%). Contrary to the present finding, Suthar (2009) reported a higher amount of TN (range 2.49–3.17%) in controls and emphasized that earthworms increase nitrogen levels by adding their excretory materials and mucus body fluid and decaying tissues of dead worms in the bin.

Decomposition rate and compost maturity depend on the C:N ratio, which is an essential factor for earthworm survival, reproduction, and other microbes in the vermicomposting process. In the final vermicomposted manures, the average amount of C:N ratio does not differ significantly ( $F_{(2, 33)} = 2.26, p > 0.05$ ) among *P. excavatus* ( $13.88 \pm 2.51$ ), *E. fetida* ( $13.14 \pm 2.1$ ) and control ( $11.69 \pm 2.99$ ). Similarly, no significant ( $F_{(3, 32)} = 1.99, p > 0.05$ ) differences were observed among the substrates (KS, CD, RS, and MX). But compared to an initial ratio of  $15.37 \pm 1.79$  to  $21.68 \pm 0.7$  depending on the substrate, C:N reduced from  $9.6 \pm 3.06$  to  $13.8 \pm 2.8$ ,  $12.75 \pm 1.00$  to  $16.84 \pm 3.6$ , and  $11.64 \pm 0.44$  to  $15.49 \pm 2.82$  in control, *P. excavatus* and *E. fetida* respectively (Table 2). In the present study, the ratio that microbes consume OC is more with little nitrogen, leading to a decline in C:N ratio. The rapid decline of organic carbon and C:N ratio during the initial stage of vermicomposting indicates the conversion of biodegradable waste into a stable end product (Gusain & Suthar, 2020; Pandit et al., 2020; Lv et al., 2018) and is also attributed to the consumption of organic matter, cellulose, and hemicellulose by earthworms (Sharma & Garg, 2019). C:N ratio from 15 to 20 is considered acceptable for vermicompost applications in agronomy (FAO, 2020).

With an initial record of Av. P (mg/kg) ranging from  $32.69 \pm 0.42$  to  $65.97 \pm 3.56$ , the total amount increased from  $41.58 \pm 0.6$  to  $86.54 \pm 0.6$  (RS > MX > CD > KS) in *P. excavatus*,  $47.66 \pm 0.56$  to  $86.55 \pm 0.74$  (RS > MX > KS > CD) in *E. fetida* and  $38.55 \pm 1.64$  to  $79.14 \pm 2.09$  (RS > MX > CD > KS) in control (Table 2) showing a mean of the range of  $59 \pm 19.27$ ,  $61.2 \pm 17.5$  and  $54.32 \pm 17.51$  in *P. excavatus*, *E. fetida*, and control respectively. Earthworm species and substrate effects on Av. P was evaluated using two-way ANOVA, and results show interaction effects of earthworm and substrate were insignificant ( $F_{(6, 24)} = 2.05, p > 0.05$ ). But simple main effects of earthworm ( $F_{(2, 24)} = 13.28, p < 0.05$ ) and substrate ( $F_{(3, 24)} = , p < 0.05$ ) have significant effects on Av. P concentration. Multiple comparisons test shows that both *P. excavatus* and *E. fetida* resulted in significantly higher concentrations ( $p < 0.05$ ). Also, Av. P in each substrate differs significantly ( $p < 0.05$ ). The increased percentage of Av. P in treatments over the initial value was recorded to be 17.89 to 20.72%, 27.21 to 32.88%, and 20.54 to 51.83% in control, *P. excavatus*, and *E. fetida*, respectively, showing a significant difference ( $F_{(2, 33)} = 5.52, p < 0.05$ ) among the treatment, and Tukey test shows, in *E. fetida* worked manure, an Av. P increased significantly ( $p < 0.05$ ) higher compared to the control. However, no significant differences were observed between *E. fetida* and *P. excavatus*.

The increased phosphorus concentration in vermicompost may be attributed to earthworm activity conducive to phosphate-dissolving bacteria in the feedstock. The activity of earthworm gut enzymes, phosphatase, formation of organic acids, and discharge of total phosphorus from a complex form of humic acid mediated by microbial activity might contribute to the increase of phosphorus in vermicompost (Gusain & Suthar, 2020; Sharma & Garg, 2018). The increased activity of phosphate solubilizing enzymes in the earthworm gut may also contribute to a higher concentration of available phosphorus (Ramanarian et al., 2019; Ghosh et al., 2018) reported that in the first 50 days, phosphatase and phytase enzymes are responsible for phosphorus mineralization. Balachandar et al. (2020) also

**Table 2** Changes in chemical characteristics of compost (manure) using earthworm species

Substrate	Before		After		Before		After			
					<i>P. excavatus</i>	<i>E. fetida</i>	Control	Control		
KS	pH									
			6.79±0.2	6.51±0.16 <sup>b</sup>	6.17±0.28 <sup>b</sup>	5.67±0.21 <sup>a</sup>	23.5±1.72	11.31±2.12 <sup>a</sup>	9.41±0.51 <sup>ab</sup>	11.34±2.22 <sup>b</sup>
			7.6±0.24	6.82±0.06 <sup>ab</sup>	7.19±0.16 <sup>a</sup>	7.29±0.15 <sup>a</sup>	23.13±1.06	16.56±1.79 <sup>a</sup>	8.46±1.23 <sup>b</sup>	16.49±2.69 <sup>a</sup>
			7.9±0.18	7.38±0.25 <sup>a</sup>	7.21±0.24 <sup>a</sup>	7.11±0.03 <sup>a</sup>	23.99±1.43	15.131.28 <sup>a</sup>	13.57±1.13 <sup>a</sup>	10.08±0.51 <sup>b</sup>
MX	TN %		7.4±0.4	7.31±0.22 <sup>a</sup>	6.88±0.09 <sup>ab</sup>	6.99±0.76 <sup>a</sup>	29.92±1.13	15.85±2.65 <sup>a</sup>	14.19±0.75 <sup>a</sup>	14.39±1.53 <sup>ab</sup>
			1.54±0.16	1.21±0.2 <sup>a</sup>	0.74±0.04 <sup>ab</sup>	0.93±0.07 <sup>ab</sup>	15.37±2.19	9.6±3.06 <sup>a</sup>	12.75±1.23 <sup>a</sup>	9.6±3.06 <sup>a</sup>
			2.07±0.03	1.5±0.1 <sup>a</sup>	0.5±0.03 <sup>b</sup>	1.06±0.01 <sup>a</sup>	21.68±0.7	10.93±2.09 <sup>a</sup>	16.84±3.6 <sup>a</sup>	10.93±2.09 <sup>a</sup>
			1.35±0.10	1.12±0.2 <sup>a</sup>	1.05±0.02 <sup>a</sup>	0.86±0.05 <sup>b</sup>	17.76±0.35	13.81±2.86 <sup>a</sup>	12.89±1.26 <sup>a</sup>	13.81±2.86 <sup>a</sup>
MX	Av. P (mg/kg)		1.86±0.01	1.31±0.23 <sup>a</sup>	1.09±0.03 <sup>a</sup>	1.07±0.05 <sup>a</sup>	16.07±0.52	12.41±3.51 <sup>a</sup>	13.03±1.03 <sup>a</sup>	12.41±3.51 <sup>a</sup>
			32.69±0.42	38.55±1.64 <sup>b</sup>	41.58±0.74 <sup>c</sup>	49.6±3.39 <sup>b</sup>	226.67±19.82	248.87±23.07	288.09±9.45	301.02±15 <sup>a</sup>
			39.64±1.82	47.07±5.1 <sup>ab</sup>	50.79±2.68 <sup>bc</sup>	47.66±0.56 <sup>b</sup>	183.36±12.81	201.24±10.25	229.24±14.11	244.4±19.75 <sup>b</sup>
			65.97±3.56	79.14±2.09 <sup>a</sup>	86.21±2.85 <sup>a</sup>	86.55±0.74 <sup>a</sup>	227.19±13.91	263.18±13	282±26.24	264.76±17.42 <sup>ab</sup>
MX	Av. K (mg/kg)		43.46±2.2	52.51±4.55 <sup>a</sup>	57.41±4.72 <sup>ab</sup>	60.87±5.2 <sup>ab</sup>	234.49±7.05	261.66±14.2	281.45±18.93	293.98±8.9 <sup>a</sup>

Data represent mean ±SD. *KS* kitchen scrap, *CD* cow dung, *RS* rice straw, *MX* mixed substrate

Mean with different superscripts (a, b, c) within the same column differ significantly by Tukey's test at  $p < 0.05$ . The Tukey test provides specific mean significant differences between the two groups

reported a 98% increase in phosphorus concentration using epigeic species, *Eudrilus euei-nae*, from green manure. At the same time, Nayak et al. (2013) reported a 29.1–46.9% increase in phosphorus concentration from vermicomposting sewage sludge.

With an initial record of Av. K (mg/kg) ranging from  $183.36 \pm 12.81$  to  $226.67 \pm 19.82$ , depending on the substrate, the total amount increased from  $201.24 \pm 10.25$  to  $263.18 \pm 13$  in control (MX>RS>KS>CD), from  $229.24 \pm 14.11$  to  $288.09 \pm 9.45$  in *P. excavatus* (KS>RS>MX>CD) and  $244.4 \pm 19.7$  to  $301 \pm 15$  in *E. fetida*, (KS>MX>RS>CD) exhibiting earthworm effect to be more (Table 2). The highest amount of Av. K was observed in *P. excavatus* ( $270.2 \pm 29.32$ ), followed by *E. fetida* ( $276.0417 \pm 27.33$ ), and control ( $243.74 \pm 29.56$ ). One-way ANOVA shows that Av. K vary significantly depending on the treatment ( $F_{(2, 33)} = 4.29$ ,  $p < 0.05$ ) and substrate used ( $F_{(3, 33)} = 11.93$ ,  $p < 0.05$ ). Multiple comparisons show that Av. K in *E. fetida* was significantly ( $p < 0.05$ ) higher compared to the control, while no significant difference was observed in comparisons to *P. excavatus* ( $p > 0.05$ ). Tukey test shows, irrespective of treatment, among the substrates, significant differences were observed among KS-CD, CD-RS, and CD-MX ( $p < 0.05$ ) (Table 2). The increased percentage of Av. K among different substrates over the initial value was recorded to be 10.24 to 27.21%, 20.07 to 27.57%, and 16.72 to 34.17% in control, *P. excavatus*, and *E. fetida*, respectively. Depending on the presence and absence of earthworms, the mean increased percentage of Av. K in the manure was significantly different ( $F_{(2, 33)} = 6.24$ ,  $p < 0.05$ ) among all the treatments, with a maximum in *E. fetida* (27.42%) followed by *P. excavatus* (24.36%) and control (14.67%). Tukey test shows that *P. excavatus* and *E. fetida* worked manures have significantly ( $p < 0.05$ ) higher percentages than the control.

Suthar (2007) reported an enhanced level of exchangeable potassium in the manure from 26.3 to 125.2% using *P. sansibaricus* and recorded higher microbial activity during the vermicomposting process of agriculture waste, farmyard manure, and urban solid waste. Zziwa et al. (2021) recorded a 74.0–81.3% increase in total potassium from vermicompost produced from pineapple waste. The enhanced level of exchangeable potassium from an insoluble state in the vermicomposted products is due to earthworms as they increase the number of microorganisms and their activity (Kaviraj & Sharma, 2003). Many earthworm gut-associated bacteria are reported to have phosphate and potassium-solubilizing bacteria. Yakkou et al. (2022) observed that out of 16 bacteria isolated from earthworm gut, six bacteria, namely *Pseudomonas aeruginosa*, *Pantoeavagans*, *Buttiauxella gaviniae*, *Raoultella planticola*, *Aeromonas sp.* *Aeromonas hydrophila* have the potential to solubilize insoluble forms of potassium. Therefore, apart from the other biochemical process, an increased Av. P and Av. K could be attributed to earthworm-associated bacteria that solubilize the bound form of P and K.

### 3.3 Changes in heavy metals concentration after vermicomposting

Earthworms directly affect heavy metals through absorption in their tissue, known as bioaccumulation (Sizmur & Hodson, 2009). Heavy metals could induce the synthesis of metallothionein isoform in earthworms' intestines that bind metal ions, forming organo-metallic ligands and thus reducing the exchangeable fractions of metals (Goswami et al., 2014). The bioaccumulation factors (BAF) of earthworms for heavy metals are in order of Cadmium (Cd) > Zinc (Zn) > Copper (Cu) > Nickel (Ni) > Lead (Pb) (Rorat et al., 2016). However, Bernard et al. (2010) argued and demonstrated that *E. fetida* could eliminate Pb but not Cd when exposed to contaminated soils.

The amount of Cu (mg/kg) in the initial stage ranged from 4.74 to 5.65, with a maximum concentration in KS followed by MX, RS, and CD, which was considerably reduced on vermicomposting (Table 3). Cu concentration in *P. excavatus* inoculated pot was reduced to  $1.49 \pm 0.03$ ,  $1.66 \pm 0.007$ ,  $1.68 \pm 0.29$ , and  $1.92 \pm 0.04$  in RS, CD, KS, and MX, respectively, with an average concentration of  $1.68 \pm 0.2$ . In contrast, in *E. fetida*, it was reduced to  $1.45 \pm 0.03$ ,  $1.55 \pm 0.31$ ,  $1.84 \pm 0.01$  and  $1.86 \pm 0.16$  in RS, KS, MX, and CD, respectively, with an average concentration of  $1.68 \pm 0.24$ . The reduction of Cu concentration in control was comparatively less, showing a mean amount of  $2.69 \pm 0.58$ . In the final manures produced, Cu concentration varies noticeably, depending on the earthworm treatment and substrates used. Two-way ANOVA was analyzed to study the effects of substrate and earthworm species on Cu concentration. The results show that the interaction effects of independent variables (substrates and treatment) were significant ( $F_{(6, 24)}=6.28$ ,  $p < 0.05$ ). Also, the main effects show earthworm species ( $F_{(2, 33)}=80$ ,  $p < 0.05$ ) and substrate ( $F_{(3, 33)}=11.04$ ,  $p < 0.05$ ) significantly affect Cu concentration.

In *P. excavatus*, the percentage reduction (%) of Cu was maximum in RS ( $70.78 \pm 1.04$ ), followed by KS ( $70.01 \pm 7.06$ ), CD ( $64.88 \pm 0.17$ ), and MX ( $64.38 \pm 1.13$ ). While in *E. fetida* treated pot, there was a gradual decline of  $72.15 \pm 7.39\%$ ,  $71.49 \pm 0.97\%$ ,  $65.72 \pm 0.18\%$ , and  $60.73 \pm 3.39\%$ , in KS, RS, MX, and CD, respectively. However, in the control pot, reduction (%) was comparatively less, as observed in RS ( $57.08 \pm 5.46$ ), CD ( $52.55 \pm 5.02$ ), MX ( $45.15 \pm 8.21$ ), and KS ( $40.3 \pm 5.87$ ). Further, the mean reduction of Cu in *P. excavatus*, *E. fetida*, and control over initial readings were  $67.51 \pm 4.32\%$ ,  $67.52 \pm 5.98\%$ , and  $48.77 \pm 8.62\%$ , respectively. One-way ANOVA shows mean reduction percentage of Cu differs significantly ( $F_{(2, 33)}=32.71$ ,  $p < 0.05$ ) in *P. excavatus*, *E. fetida*, and control. Multiple comparisons test show no significant differences ( $p > 0.05$ ) between the two earthworm species, but the amount of Cu in earthworm in worked manures were significantly ( $p < 0.05$ ) lower than the control. Lv et al. (2016) reported a significant reduction of exchangeable Cu during vermicomposting. They emphasized that earthworm bodies might take up the heavy metals, which leads to a decrease in Cu concentration. Similarly, Suthar et al. (2014) reported that vermistabilization significantly reduced the amount of Cu (68.8–88.4%) and demonstrated that bioaccumulation of heavy metals by earthworms was in the order  $Cd > Cr > Pb > Cu$ . Pattanaik & Reddy (2011) reported that earthworms remediate heavy metals from the waste by bioaccumulating in their body tissue, resulting in a decline in its concentration in vermicomposted manure. Differences in the bioaccumulation potential of earthworms could be due to variations in earthworm species.

Having the initial amount of Fe (mg/kg) ranging from  $258.66 \pm 2.68$  in KS,  $220.64 \pm 5.54$  in CW,  $179.93 \pm 2.5$  in RS,  $127.98 \pm 16.38$ , the concentration was reduced to  $97.25 \pm 1.89$  to  $151.86 \pm 2.14$ ,  $112.76 \pm 5.57$  to  $146.32 \pm 3.02$  and  $105.4 \pm 15.83$  to  $163.66 \pm 13.57$  in *P. excavatus*, *E. fetida* and control pots respectively in all substrates (Table 3). The final Fe concentration in control, *P. excavatus*, and *E. fetida* were  $141.85 \pm 25.34$ ,  $125.53 \pm 20.70$ , and  $133.89 \pm 14.03$ . Unlike other parameters, Fe concentration in different earthworm treatments does not differ significantly ( $F_{(2, 33)}=1.88$ ,  $p > 0.05$ ). However, significant ( $F_{(3, 33)}=21.52$ ,  $p < 0.05$ ) differences among the substrate were observed.

The percentage reduction (%) of Fe with *P. excavatus* was maximum in CD ( $55.47 \pm 2.67$ ) followed by KS ( $53.34 \pm 0.59$ ), MX ( $23.21 \pm 9.49$ ), and RS ( $15.6 \pm 0.14$ ). While in *E. fetida* treated pot, there was a decline of  $11.3 \pm 7.2\%$ ,  $26.1 \pm 0.94\%$ ,  $33.62 \pm 3.03\%$ , and  $44.5 \pm 0.43\%$  in MX, RS, CD, and KS, respectively. In the control pot, reduction (%) was comparatively less, as observed in RS ( $9.07 \pm 6.74$ ), MX ( $17.75 \pm 4.02$ ), CD ( $33.27 \pm 5.98$ ), and KS ( $41.51 \pm 3.85$ ). Further, the highest mean reduction (%) of Fe was observed in *P. excavatus*, *E. fetida*, and control with  $36.9 \pm 22.11\%$ ,  $28.88 \pm 13.03\%$ ,

**Table 3** Changes in heavy metals concentration obtained from control and earthworm treatment after vermicomposting

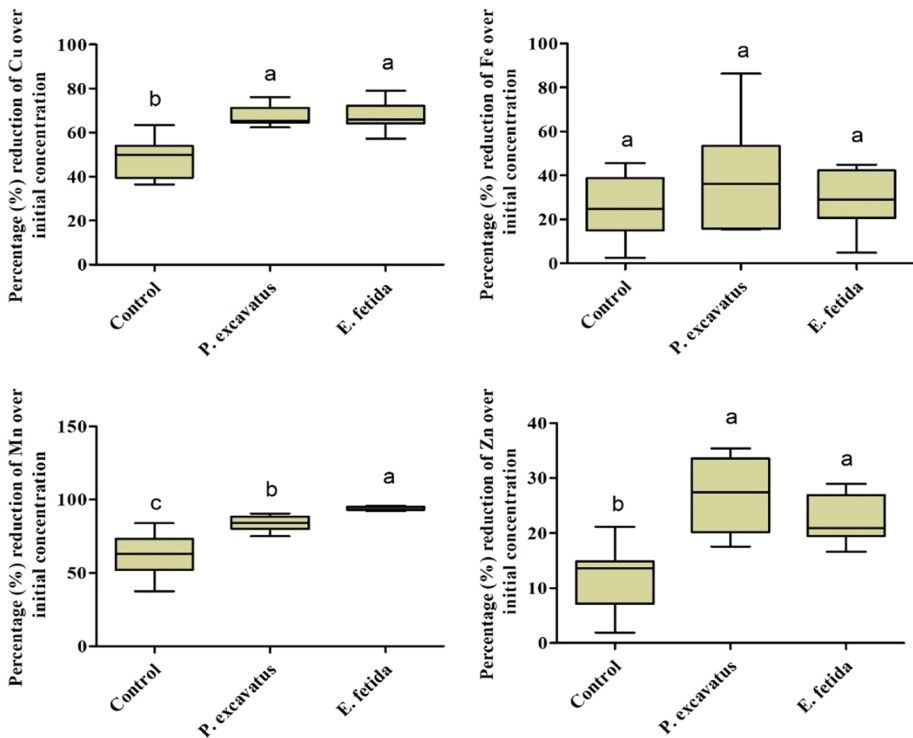
Substrate	Before		After		Before		After	
	Control	<i>E. fetida</i>	Control	<i>E. fetida</i>	Control	<i>E. fetida</i>	Control	<i>E. fetida</i>
	Cu (mg/kg)							
KS	5.65 ± 0.36 <sup>a</sup>	4.74 ± 0.04 <sup>b</sup>	3.36 ± 0.26 <sup>a</sup>	1.68 ± 0.29 <sup>ab</sup>	1.55 ± 0.31 <sup>a</sup>	258.66 ± 2.68 <sup>a</sup>	151.33 ± 11.2 <sup>ab</sup>	143.53 ± 0.36 <sup>ab</sup>
CD	4.74 ± 0.04 <sup>b</sup>	5.1 ± 0.06 <sup>ab</sup>	2.25 ± 0.24 <sup>a</sup>	1.66 ± 0.00 <sup>ab</sup>	1.86 ± 0.16 <sup>a</sup>	220.64 ± 5.54 <sup>ab</sup>	147 ± 9.53 <sup>ab</sup>	146.32 ± 3.02 <sup>a</sup>
RS	5.1 ± 0.06 <sup>ab</sup>	5.39 ± 0.05 <sup>ab</sup>	2.19 ± 0.27 <sup>a</sup>	1.49 ± 0.03 <sup>c</sup>	1.45 ± 0.03 <sup>a</sup>	179.03 ± 2.5 <sup>ab</sup>	163.66 ± 13.57 <sup>a</sup>	132.95 ± 0.16 <sup>ab</sup>
MX	5.39 ± 0.05 <sup>ab</sup>		2.95 ± 0.43 <sup>a</sup>	1.92 ± 0.04 <sup>a</sup>	1.84 ± 0.01 <sup>a</sup>	127.98 ± 16.38 <sup>b</sup>	105.4 ± 15.83 <sup>b</sup>	112.76 ± 5.57 <sup>b</sup>
	Mn (mg/kg)							
KS	56.02 ± 1.33 <sup>a</sup>		12.24 ± 2.8 <sup>a</sup>	5.54 ± 0.33 <sup>b</sup>	5.34 ± 0.46 <sup>ab</sup>	15.17 ± 0.64 <sup>a</sup>	12.80 ± 0.91 <sup>a</sup>	10.32 ± 0.26 <sup>ab</sup>
CD	51.22 ± 2.05 <sup>a</sup>		27.66 ± 6.15 <sup>a</sup>	11.39 ± 1.19 <sup>a</sup>	7.32 ± 0.31 <sup>a</sup>	14.1 ± 0.06 <sup>a</sup>	13.51 ± 0.46 <sup>a</sup>	10.99 ± 0.26 <sup>ab</sup>
RS	54.11 ± 1.49 <sup>a</sup>		18.81 ± 2.88 <sup>a</sup>	7.43 ± 0.44 <sup>ab</sup>	4.54 ± 0.45 <sup>b</sup>	14.54 ± 0.38 <sup>a</sup>	12.1 ± 0.61 <sup>a</sup>	9.49 ± 0.37 <sup>b</sup>
MX	56.01 ± 0.88 <sup>a</sup>		21.88 ± 6.91 <sup>a</sup>	9.90 ± 0.20 <sup>ab</sup>	5.04 ± 0.24 <sup>ab</sup>	14.83 ± 0.35 <sup>a</sup>	13.22 ± 0.23 <sup>a</sup>	11.91 ± 0.13 <sup>a</sup>

Data represent mean ± SD. KS kitchen scrap, CD cow dung, RS rice straw, MX mixed substrate

Mean with different superscripts (a, b, c) within the same column differ significantly by Tukey's test at  $p < 0.05$ . The Tukey test provides specific mean significant differences between the two groups

and  $25.4 \pm 14.02\%$  but no significant ( $F_{(2, 33)} = 1.46, p > 0.05$ ) differences have resulted among the treatment (Fig. 4). Suthar & Singh (2008) also reported 13.1 to 19.9% reduction of Fe, and showed that vermicomposting significantly reduces heavy metals compared to experimental compost without earthworms and further confirmed the decrease in heavy metals was attributed to earthworms activity. Likewise, Hobbelen et al. (2006) reported that heavy metal availability in earthworm tissues increases while its concentration decreases in vermicompost.

Initial concentration of Mn (mg/kg) ranged from  $51.22 \pm 2.05$  to  $56.02 \pm 0.88$  in the different substrates, with the maximum amount in CD, followed by RS, KS, and MX (Table 2). However, the concentration was substantially decreased at the end of the experiment in all treatments (Table 3). With the treatment of *P. excavatus*, the Mn concentration was reduced to  $5.54 \pm 0.33$ – $11.39 \pm 1.19$  showing the maximum reduction in KS (90.08%), followed by RS (86.26%), MX (82.32%), and CD (77.74%). In *E. fetida* treated pot also, there was a decline of 95.45%, 94.65%, 94.95%, and 92.67% in RS, KS, MX, and CD, respectively, having a final concentration ranges of  $4.54 \pm 0.45$  to  $7.32 \pm 0.31$ . In the control pot also, Mn was reduced considerably ( $12.24 \pm 2.8$  to  $27.66 \pm 6.15$ ), highlighting the maximum reduction (%) in KS (78.06  $\pm$  5.47%) followed by RS (65.3  $\pm$  4.63%), MX (61.05  $\pm$  11.76%), and CD (46.17  $\pm$  10.49%). The average amount of Mn in *P. excavatus*, *E. fetida*, and control were  $8.56 \pm 2.41$ ,  $5.56 \pm 1.14$ , and  $20.14 \pm 7.22$ , respectively. Two-way



**Fig. 4** Percentage reduction of heavy metal concentration over initial value in earthworms worked manure and control manure. Mean with different superscripts (a, b, c) among the treatment differ significantly ( $p < 0.05$ ) by Tukey's test at a 95% confidence level

ANOVA shows that the interaction effect of earthworm species and substrate impact Mn concentration significantly ( $F_{(6, 13)}=2.99$ ,  $p<0.05$ ). While the main effects show earthworm species treatment ( $F_{(2, 33)}=81.92$ ,  $p<0.05$ ) and substrate used ( $F_{(3, 33)}=11.09$ ,  $p<0.05$ ) also have a significant effect on Mn concentration. Tukey test shows Mn concentration in *P. excavatus* and *E. fetida* were significantly ( $p<0.05$ ) lower compared to the control, however, no significant ( $p>0.05$ ) differences were observed between the two earthworm species. Further, mean variation in the reduction percentage of Mn in *E. fetida* ( $94.43 \pm 0.1\%$ ), *P. excavatus* ( $84.1 \pm 0.95\%$ ), and control ( $62.64 \pm 3.56\%$ ) over initial were significantly ( $F_{(2, 33)}=42.73$ ,  $p<0.05$ ) different and showed better efficiency of earthworm species.

The expressively higher percentage reduction of Mn in the earthworm-treated pot could be due to the absorption of most available fractions of Mn through the epithelial tissue of earthworms during vermistabilizations (Singh & Kalamdhad, 2013) also reported a 42.6 to 84.6% reduction in heavy metals during vermicomposting. However, in contrast to the present study, Soobhany et al. (2015) have shown that vermicomposting increases Mn concentrations considerably and asserted that formations of the organically bound complex rather than augmentation in the total content might lead to increased Mn concentrations.

The initial concentration of Zn (mg/kg) ranged from  $14.1 \pm 0.06$  to  $15.17 \pm 0.64$  in different substrates, and the concentration of Zn at the end of vermicomposting was found to be  $9.49 \pm 0.37$  to  $11.91 \pm 0.13$ ,  $10.53 \pm 0.18$  to  $12.08 \pm 0.03$  and  $12.1 \pm 0.61$  to  $13.51 \pm 0.46$  in *P. excavatus*, *E. fetida* and control respectively (Table 3). The average amount of Zn in *P. excavatus*, *E. fetida*, and control were  $10.68 \pm 0.95$ ,  $11.31 \pm 0.61$ , and  $12.91 \pm 0.75$  showing significant ( $F_{(2, 33)}=25.5$ ,  $p<0.05$ ) differences among earthworm treatment and control. Multiple comparisons test indicate no significant ( $p>0.05$ ) differences between two earthworm species in minimizing the Zn concentration but both *P. excavatus* and *E. fetida* showed significant ( $p<0.05$ ) effects on Zn. In *P. excavatus*, maximum reduction (%) of Zn concentration was recorded in RS ( $34.75 \pm 1.02\%$ ) followed by KS ( $31.95 \pm 1.36\%$ ), CD ( $22.02 \pm 2.25\%$ ), and MX ( $19.68 \pm 1.17\%$ ), it was noticed to be comparatively less in *E. fetida* treated substrates of RS ( $27.54 \pm 1.2\%$ ), KS ( $24.35 \pm 4.68\%$ ), CD ( $20.80 \pm 0.2\%$ ), and MX ( $18.54 \pm 1.71\%$ ). In control, the maximum reduction was observed in RS > KS > MX > CD with  $15.68 \pm 2.99$ ,  $16.75 \pm 3.82$ ,  $10.84 \pm 3.52$ , and  $4.22 \pm 0.34$ . Analysis of variance shows significant variations in the mean reduction percentage of Zn were observed depending on the treatment ( $F_{(2, 33)}=22.26$ ,  $p<0.05$ ) and substrate ( $F_{(3, 33)}=4.63$ ,  $p<0.05$ ). Tukey test shows reduction (%) of Zn concentration in *P. excavatus* and *E. fetida* were significantly higher ( $p<0.05$ ) compared to the control, while no significant difference was observed between earthworms ( $p>0.05$ ) (Table 3; Fig. 4).

While studying the remediation of heavy metals from urban waste using earthworms, Pattanik & Reddy (2011) observed a significant number of metals increase in earthworm tissue, and 56% of Zn was removed from the biodegradable waste within 60th days. Dominguez et al. (1997) also confirmed that in 60 days, the bioavailability of heavy metals (Zn and Cu) decreased by 35–55%. Pattanaik & Reddy (2011) reported that earthworms remediate heavy metals from the waste by absorbing their body, resulting in a decreased concentration in the manures. The present findings indicate the potentiality of *P. excavatus* and *E. fetida* to reduce heavy metal toxicity and remediate the polluted landscape. Among the different ecological categories of earthworms, epigeic earthworm such as *E. fetida* has shown better bioaccumulation capability of heavy metals compared to endogeic and anecic earthworms (Turgay et al., 2011). It is suggested that phosphorus treatment significantly reduces the bioavailability of metals (Cd, Zn, and Pb), probably due to the formation of metal phosphate complexes in the soil (Maenpaa et al.,



2002). Similarly, due to the joint action of earthworms and bacteria (primarily PSB), an increased amount of phosphorus in the vermicomposted manure might decrease heavy metal availability.

## 4 Conclusion

In the present study, a high degree of organic waste degradation and nutrient stabilization was achieved using *P. excavatus* and *E. fetida*, demonstrating that vermicomposting delivers sustainable, environmentally friendly, and cost-effective means of waste management. Nutrient stabilization was observed through a reduced C:N ratio and increased Av. P and Av. K in the final vermicomposted manures. The high percentage reduction of Cu, Fe, Mn, and Zn was observed in both *P. excavatus*, and *E. fetida* worked manures. In addition to waste management, the higher increased percentage of macronutrients, minimizing the vast quantity of heavy metals by earthworms, indicate their potential application in the remediation and mitigation of nutrient-depleted and polluted soil.

It can be concluded that with sufficient macronutrients (N, Av. P, and Av. K) and reduced concentrations of heavy metals (Cu, Fe, Mn, and Zn) resulted in the present study; vermicompost can be used as a soil conditioner in agronomy. Overall, agricultural waste, domestic waste, and cow dung are suitable substrates for earthworms to work on and avoid the loss of organic waste biomass and environmental pollution. However, with better technique and equipment, the present study can be reinforced by the assessment, isolation, and characterization of microbes associated with earthworms' body that has the potential to solubilize the nutrient available in the soil and substrates. Also, the current study was carried out in pre conditioned environment and small-scale experiment. Therefore, it is desirable to study the potential application of *P. excavatus* and *E. fetida* in large-scale waste degradation for other biodegradable wastes in an uncontrolled environment. Such work can lead to a better indulgence in eco-friendly waste management and the production of organic fertilizer that can be used as an alternative to synthetically produced fertilizers with a minimal negative impact on the soil system.

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**Data availability** All data generated or analysed during this study are included in this article.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

## References

- Alemayehu, Y. A., Asfaw, S. L., & Terfie, T. A. (2022a). Hydrolyzed urine as a nutrient and condition corrector for enhanced coffee pulp composting. *Environmental Technology & Innovation*, 28, 703.
- Alemayehu, Y. A. (2022b). Hydrolyzed urine for enhanced valorization and toxicant degradation of wet coffee processing wastes: implications for soil contamination and health risk reductions. *Journal of Environmental Management*, 307, 114536.
- Balachandar, R., Baskaran, L., Yuvaraj, A., Thangaraj, R., Subbaiya, R., Ravindran, B., Chang, S. W., & Karmegam, N. (2020). Enriched pressmud vermicompost production with green manure plants using *Eudrilus eugeniae*. *Bioresource Technology*, 299, 122578.
- Bernard, F., Brulle, F., Douay, F., Lemiere, S., Demuynck, S., & Vandenbulcke, F. (2010). Metallic trace element body burdens and gene expression analysis of biomarker candidates in *Eisenia fetida*, using an “exposure/depuration” experimental scheme with field soils. *Ecotoxicology and Environmental Safety*, 73, 1034–1045.
- Bhagat, T. S., Lokhande, R. S., Petha, N. H., & Chandorkar, J. G. (2022). Vermicomposting via pre-composting of eucalyptus leaves for proper waste management and sustainable agriculture growth. *Communications in Soil Science and Plant Analysis*, 54(6), 785–804.
- Borang, B., Sharma, Y. K., & Sharma, S. K. (2016). Effect of various substrates on performance of earthworm and quality of vermicompost. *Annals of Plant and Soil Research*, 18(1), 37–42.
- Bray, R. H., & Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Science*, 59(1), 39–46.
- Chatterjee, D., Kuotsu, R., James Kikon, Z., Sarkar, D., Ao, M., Ray, S. K., & Deka, B. C. (2016). Characterization of vermicomposts prepared from agricultural solid wastes in north eastern hill region of Nagaland, India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 86(4), 823–833.
- Devi, C., & Khwairakpam, M. (2020). Bioconversion of *Lantana camara* by vermicomposting with two different earthworm species in monoculture. *Bioresource Technology*, 296, 122308.
- Doaemo, W., Dhiman, S., Borovskis, A., Zhang, W., Bhat, S., Jaipuria, S., & Mirzi, B. M. (2021). Assessment of municipal solid waste management system in Lae city, Papua New Guinea in the context of sustainable development. *Environment Development and Sustainability*, 23, 18509–18539.
- Dominguez, J., Briones, M. J. I., & Mato, S. (1997). Effect of the diet on growth and reproduction of *Eisenia andrei* (Oligochaeta, Lumbricidae). *Pedobiologia*, 41, 566–576.
- Dominguez, J., & Edwards, C. A. (2011). *Biology and ecology of earthworm species used for vermicomposting. Vermiculture technology: earthworms, organic waste and environmental management* (pp. 27–40). Boca Raton: CRC Press.
- Edwards, C. A., & Bohlen, P. J. (1996). *Biology and ecology of earthworm* (p. 46). London: Chapman and Hall.
- Fahmy, T. Y. A., Fahmy, Y., Mobarak, F., El-Sakhawy, M., & Abou-Zeid, R. E. (2020). Biomass pyrolysis: past, present, and future. *Environment Development and Sustainability*, 22, 17–32. <https://doi.org/10.1007/s10668-018-0200-5>
- Fahmy, T. Y. A., & Mobarak, F. (2013). Advanced binderless board-like green nanocomposites from unbarked cotton stalks and mechanism of self-bonding. *Cellulose*, 20(3), 1453.
- Fahmy, Y., Fahmy, T. Y. A., Mobarak, F., El-Sakhawy, M., & Fadl, M. (2017). Agricultural residues (wastes) for manufacture of paper, board, and miscellaneous products: background overview and future prospects. *International Journal of ChemTech Research*, 10, 424–448.
- FAO (2020). A protocol for measurement, monitoring, reporting and verification of soil organic carbon in agricultural landscapes—GSOC-MRV Protocol. Rome. <https://doi.org/10.4060/cb0509en>
- Ghosh, S., Goswami, A. J., Ghosh, G. K., & Pramanik, P. (2018). Quantifying the relative role of phytase and phosphatase enzymes in phosphorus mineralization during vermicomposting of fibrous tea factory waste. *Ecological Engineering*, 116, 97–103.
- Goswami, L., Sarkar, S., Mukherjee, S., Das, S., Barman, S., Raul, P., Bhattacharyya, N. C., Mandal, S., & Bhattacharya, S. S. (2014). Vermicomposting of tea factory coal ash: metal accumulation and metallothionein response in *Eisenia fetida* (Savigny) and *Lampito mauritii* (Kinberg). *Bioresource Technology*, 166, 96–102.
- Gusain, R., & Suthar, S. (2020). Vermicomposting of duckweed (spirodelapolyrhiza) by employing *Eisenia fetida*: changes in nutrient contents, microbial enzyme activities and earthworm biodynamics. *Bioresource Technology*, 311, 123585.
- Hanway, J. J., & Heidel, H. (1952). Soil analysis methods as used in Iowa state college soil testing laboratory. *Iowa Agriculture*, 57, 1–31.

- Hasan, M., Ahmed, S., Marimuthu, N., & Deuti, K. (2022). Notes on the Identification of Earthworm Species Suitable for Vermicomposting Purposes in India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 1–13.
- Hobbelen, P. H. F., Koolhaas, J. E., & van Gestel, C. A. M. (2006). Bioaccumulation of heavy metals in the earthworms *Lumbricus rubellus* and *Aporrectodea caliginosa* in relation to total and available metal concentrations in field soils. *Environmental Pollution*, 144, 639–646.
- Huntley, S., & Ansari, A. (2021). Vermicomposting evaluation of different combinations of organic waste using *Perionyx excavatus*. *The International Journal of Recycling of Organic Waste in Agriculture*, 10(3), 287–295.
- ICAR. (2020). *Creating wealth from agricultural waste* (p. 172). Indian Council of Agricultural Research.
- Javadinejad, S., Eslamian, S., & Ostad-Ali-Askari, K. (2019). Investigation of monthly and seasonal changes of methane gas with respect to climate change using satellite data. *Applied Water Science*, 9, 180.
- Jayakumar, M., Eman, A. N., Subbaiya, R., Ponraj, M., Kumar, K. K. A., Muthusamy, G., Kim, W., & Karmegam, N. (2022). Detoxification of coir pith through refined vermicomposting engaging *Eudrilus eugeniae*. *Chemosphere*, 291, 132675.
- Kaladhar, D. S., & Srinivasan, T. (2022). Production of commercial products by vermiculture and vermicomposting. *Biotechnology for waste biomass utilization* (pp. 231–249). Apple Academic Press.
- Kaviraj, & Sharma, S. (2003). Municipal solid waste management through vermicomposting employing exotic and local species of earthworms. *Bioresource Technology*, 90, 169–173.
- Letcher, T. M., & Vallero, D. A. (2019). *Waste: a handbook for management*. Academic Press.
- Li, T., Lu, M., Xu, B., Chen, H., Li, J., Zhu, Z., Yu, M., Zheng, J., Peng, P., & Wu, S. (2022). Multiple perspectives reveal the gut toxicity of polystyrene microplastics on *Eisenia fetida*: insights into community signatures of gut bacteria and their translocation. *Science of the Total Environment*, 838, 156352.
- Lindsay, W. L., & Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3), 421–428.
- Lirikum., Kakati, L. N., Thyug, L., & Mozhui, M. (2022). Vermicomposting: an eco-friendly approach for waste management and nutrient enhancement. *Tropical Ecology*, 63, 325–337.
- Lv, B., Xing, M., & Yang, J. (2016). Speciation and transformation of heavy metals during vermicomposting of animal manure. *Bioresource Technology*, 209, 397–401.
- Lv, B., Zhang, D., Cui, Y., & Yin, F. (2018). Effects of C/N ratio and earthworms on greenhouse gas emissions during vermicomposting of sewage sludge. *Bioresource Technology*, 268, 408–414.
- Maenpaa, K. A., Kukkonen, J. V. K., & Lydy, M. J. (2002). Remediation of heavy metal-contaminated soils using phosphorus: evaluation of bioavailability using an earthworm bioassay. *Archives of Environmental Contamination and Toxicology*, 43(4), 0389–0398.
- Mago, M., Yadav, A., Gupta, R., & Garg, V. K. (2021). Management of banana crop waste biomass using vermicomposting technology. *Bioresource Technology*, 326, 124742.
- Nayak, A. K., Varma, V. S., & Kalamdhad, A. S. (2013). Effects of various C/N ratios during vermicomposting of sewage sludge using *Eisenia fetida*. *Journal of Environmental Science and Technology*, 6(2), 63–78.
- Negi, R., & Suthar, S. (2018). Degradation of paper mill wastewater sludge and cow dung by brown-rot fungi *Oligoporus placenta* and earthworm (*Eisenia fetida*) during vermicomposting. *Journal of Cleaner Productions*, 201, 842–852.
- Ostad-Ali-Askari, K. (2022). Management of risks substances and sustainable development. *Applied Water Science*, 12(4), 1–23.
- Pandit, L., Sethi, D., Pattanayak, S. K., & Nayak, Y. (2020). Bioconversion of lignocellulosic organic wastes into nutrient rich vermicompost by *Eudrilus eugeniae*. *Bioresource Technology Reports*, 12, 100580.
- Pattanik, S., & Reddy, M. V. (2011). Heavy metals remediation from urban wastes using three species of earthworm (*Eudrilus eugeniae*, *Eisenia fetida* and *Perionyx excavatus*). *Journal of Environmental Chemistry and Ecotoxicology*, 3(14), 345–356.
- Paul, J. J., Karmegam, N., & Daniel, T. (2011). Municipal solid waste (MSW) vermicomposting with an epigeic earthworm, *Perionyx ceylanensis* Mich. *Bioresource Technology*, 102(12), 6769–6773.
- Rajkhowa, D. J., Bhattacharyya, P. N., Sarma, A. K., & Mahanta, K. (2015). Diversity and distribution of earthworms in different soil habitats of Assam, north-east India, an Indo-Burma biodiversity hotspot. *Proceedings of the National Academy of Science India Section B Biological Science*, 85(2), 389–396.
- Ramanarian, Y. I., Ansari, A. A., & Ori, L. (2019). Vermicomposting of different organic materials using the epigeic earthworm *Eisenia foetida*. *The International Journal of Recycling of Organic Waste in Agriculture*, 8(1), 23–36.
- Reinecke, A. J., & Hallatt, L. (1989). Growth and cocoon production of *Perionyx excavatus* (Oligochaeta). *Biology of Fertile Soils*, 8, 303–306.

- Reinecke, A. J., Viljoen, S. A., & Saayman, R. J. (1992). The suitability of *Eudrilus eugeniae*, *Perionyx excavatus* and *Eisenia fetida* (Oligochaeta) for vermicomposting in Southern Africa in terms of their temperature requirements. *Soil Biology and Biochemistry*, 24, 1295–1307.
- Rini, J., Deepthi, M. P., Saminathan, K., Narendhirakannan, R. T., Karmegam, N., & Kathireswari, P. (2020). Nutrient recovery and vermicompost production from livestock solid wastes with epigeic earthworms. *Bioresource Technology*, 313, 123690.
- Rorat, A., Suleiman, H., Grobelak, A., Grosser, A., Kacprzak, M., Płytycz, B., & Vandenbulcke, F. (2016). Interactions between sewage sludge-amended soil and earthworms—comparison between *Eisenia fetida* and *Eisenia andrei* composting species. *Environmental Science and Pollution Research*, 23(4), 3026–3035.
- Sharma, K., & Garg, V. K. (2018). Vermicomposting: a green technology for organic waste management. In R. Singhanian, R. Agarwal, R. Kumar, & R. Sukumaran (Eds.), *Waste to wealth, energy, environment and sustainability* (pp. 199–235). Singapore: Springer.
- Sharma, K., & Garg, V. K. (2019). Vermicomposting of waste: a zero-waste approach for waste management. In J. Mohammad, K. Taherzadeh, J. Bolton Wong, & A. Pandey (Eds.), *Sustainable resource recovery and zero waste approaches* (pp. 133–164). Elsevier.
- Sharma, K., & Garg, V. (2017). Management of food and vegetable processing waste spiked with buffalo waste using earthworms (*E. fetida*). *Environmental Science and Pollution Research*, 24, 7829–7836.
- Singh, J., & Kalamdhad, A. S. (2013). Reduction of bioavailability and leachability of heavy metals during vermicomposting of water hyacinth. *Environmental Science and Pollution Research*, 20(12), 8974–8985.
- Sizmur, T., & Hodson, M. E. (2009). Do earthworms impact metal mobility and availability in soil? A review. *Environmental Pollution*, 157(7), 1981–1989.
- Soobhany, N., Mohee, R., & Garg, V. K. (2015). Comparative assessment of heavy metals content during the composting and vermicomposting of municipal solid waste employing *Eudrilus eugeniae*. *Waste Management*, 39, 130–145.
- Sun, M., Chao, H., Zheng, X., Deng, S., Ye, M., & Hu, F. (2020). Ecological role of earthworm intestinal bacteria in terrestrial environments: A review. *Science of the Total Environment*, 740, 140008.
- Suthar, S. (2007). Production of vermifertilizer from guar gum industrial waste by using composting earthworm *Perionyx sansibaricus* (Perrier). *The Environmentalist*, 27(3), 329–335.
- Suthar, S. (2009). Bioremediation of agricultural wastes through vermicomposting. *Bioremediation Journal*, 13(1), 21–28.
- Suthar, S., Sajwan, P., & Kumar, K. (2014). Vermiremediation of heavy metals in wastewater sludge from paper and pulp industry using earthworm. *Eisenia fetida Ecotoxicology Environmental Safety*, 109, 177–184.
- Suthar, S., & Singh, S. (2008). Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. *Science of the Total Environment*, 394, 237–243.
- Thomas, G. V., Mathew, A. E., Baby, G., & Mukundan, M. K. (2019). Bioconversion of residue biomass from a tropical homestead agro-ecosystem to value added vermicompost by *Eudrilus* species of earthworm. *Waste and Biomass Valorization*, 10(7), 1821–1831.
- Turgay, O. C., Kizilkaya, R., Karaca, A., & Cetin, S. C. (2011). Detoxification of Heavy Metals Using Earthworms. In I. Sherameti & A. Varma (Eds.), *Detoxification of Heavy Metals*. Springer.
- Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, 37(1), 29–38.
- Wang, L. K., Wang, M. H. S., Hung, Y. T., Li, K. H., Aziz, H. A., Yusoff, M. S., & Palaniandy, P. (2022). Vermicomposting process for Treating Agricultural and Food Wastes. *Waste Treatment in the Biotechnology, Agricultural and Food Industries* (pp. 173–203). Springer.
- Wang, Y., Han, W., Wang, X., Chen, H., Zhu, F., Wang, X., & Lei, C. (2017). Speciation of heavy metals and bacteria in cow dung after vermicomposting by the earthworm, *Eisenia fetida*. *Bioresource Technology*, 245, 411–418.
- Yadav, A., & Garg, V. K. (2016). Vermiconversion of biogas plant slurry and parthenium weed mixture to manure. *International Journal of Recycling of Organic Waste in Agriculture*, 5, 301–309.
- Yakkou, L., Houida, S., Bilen, S., Kaya, L. O., Raouane, M., Amghar, S., & El, Harti, A. (2022). Assessment of earthworm (*Aporrectodea molleri*)’s coelomic fluid-associated bacteria on different plant growth-promoting traits and maize germination and seedling growth. *Biocatalysis and Agricultural Biotechnology*, 42, 102341.
- Zhi-Wei, S., Tao, S., Wen-Jing, D., & Jing, W. (2019). Investigation of rice straw and kitchen waste degradation through vermicomposting. *Journal of Environmental Management*, 243, 269–272.

- Zhu-Barker, X., Bailey, S. K., Burger, M., & Horwath, W. R. (2017). Greenhouse gas emissions from green waste composting windrow. *Waste Management*, *59*, 70–79.
- Zziwa, A., Jjagwe, J., Kizito, S., Kabenge, I., Komakech, A. J., & Kayondo, H. (2021). Nutrient recovery from pineapple waste through controlled batch and continuous vermicomposting systems. *Journal of Environmental Management*, *279*, 111.

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